

Research paper

**Physiological responses of *Ulmus minor* Mill. to ozone, carbon monoxide, and nitrogen dioxide in regions with different levels of atmospheric pollutants in Iran**

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**Abstract**

The present study was aimed to investigate the physiological responses of the leaves of *Ulmus minor* to air pollutants such as ozone, carbon monoxide, and nitrogen dioxide to verify the ability of this species to resist the air pollutants. The leaves of *Ulmus minor* Mill. were collected from one location (Chaloos) in the Mazandaran province with lower air pollution and three locations in the Tehran province (Tajrish, Park Laleh, and Park Shahr) with higher air pollutions. The leaves were randomly collected from the middle part of the crowns in each sampling location with five replications and the growth and physiological characters were measured. The climate factors of the sampling locations were also evaluated. The results showed that dry matter, protein, chlorophyll a, anthocyanin, and activity of the peroxidase enzyme in the plants grown in Park Shahr with higher amount of air pollutants was almost 35, 50, 30, 50, and 40 percent higher than those of the plants grown in Chaloos with the lower amount of air pollutants, respectively. The number and intensity of peroxidase isoenzyme bands were significantly higher in the leaves of plants collected from the locations in Tehran province. However, the prominent role of rainfall on the physiological responses of *Ulmus minor* cannot be ignored. It seems that *Ulmus minor* is a resistant tree to air pollutants and it might be considered as a biological filter in removing gaseous pollutants in areas like Tehran city to improve air quality.

**Keywords:** Air pollution; Antioxidants; Climate factors; Resistance

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**Introduction**

Trees are affected by several stresses in urban environments especially by air pollutants which can be contributed to either short-term (acute) or long-term (chronic) damage (Jacobson & Hill, 1970). Indeed, acute damage causes immediate plant tissue damage and subsequently death while chronic damage inhibits plant function without causing tissue death (Chen *et al.* 2009). The main hazardous air pollutants are carbon monoxide (CO), nitrogen dioxide (NO<sub>2</sub>), ozone (O<sub>3</sub>), and sulfur dioxide (SO<sub>2</sub>) fine suspended particulates with a size of fewer than 10 microns (Samet *et al.* 2000). Some pollutants such as CO and SO<sub>2</sub> are

emitted directly into the atmosphere and are known as primary pollutants. SO<sub>2</sub> as a primary cause of acid deposition is a result of fossil fuel burning containing sulfur for electricity generation and also the processing of steel and other ores (Holman 1999). Industrial processes and CO emission are the sources of non-transportation fuel combustion (Han and Naehar 2006). O<sub>3</sub> as a secondary pollutant is formed in a chemical reaction with other pollutants and atmospheric gases. O<sub>3</sub> is created by methane, non-methane volatile organic compounds, and the photochemical oxidation of CO, in the presence of reactive nitrogen oxides (Zhang *et al.* 2016).

Some pollutants like NO<sub>2</sub> and some particulate matter belong to both primary and secondary pollutants (Holman 1999). Fossil fuel combustion and automobile exhaust gas are prominent sources of NO<sub>2</sub> (Swietlicki *et al.* 1996). The total suspended particulates (TPS) are generated by anthropogenic sources (De Kok 1990) and motor vehicles (diesel exhaust) (Jiang *et al.* 2016), however, polycyclic aromatic hydrocarbons and their derivatives also can increase the toxic potency of TPS (De Kok 1990).

The content of pollutants and the duration of trees exposed to pollutants are in turn influenced by climate conditions including temperature, humidity, and rainfall (Agbaire and Esiefarienrhe 2009; Farhadi *et al.* 2018). Temperature and rainfall play an important role in the oxidation rate, decreasing the content and even removal of pollutants including O<sub>3</sub>, NO, SO<sub>2</sub>, and carbon particles (Yoo *et al.* 2014). Temperature affects the vertical movement of air pollutants. If warm air is close to the soil surface causes pollutants to rise vertically and pollutants will be diluted and distributed by the upper-level winds without contacting trees. However, cool air at the soil surface especially beneath warmer air limits the movement of the pollutants which can be resulted in enhancing the phytotoxic levels of the pollutants. Also, humidity affects the relative abundance of hydroxyl radicals that influence the chemical process that controls the concentration of gaseous pollutants (Beirle *et al.* 2011). In addition, wind speed and direction affect the transport and dilution of pollutants (Akyuz and Cabuk 2009).

Trees can be considered as one of the best indicators for research on environmental stresses due to their long lifespan (Dobbertin 2005). Different trees exhibit different sensitivities to air pollution, and under the influence of harmful pollutants, the growth will be reduced and the physiological function of plants disturbed, however, they can enhance their chances of survival against environmental stresses with different defense mechanisms (Bosu and Wagner 2014).

*Ulmus minor* is a deciduous small or medium-sized tree with a wide crown that is used as an ornamental and roadside tree (Mozaffarian 2005). In addition, *U. minor* as a medicinal plant inhibits inflammation in several body organs (Li *et al.* 2017), acts as an anti-oxidant, and has anti-bacterial properties (Khan *et al.* 2009). Also, it is used as firewood, fodder supplier, and it is interested in the wood industry (Mozaffarian 2005).

Air pollution can directly affect plants via leaves or indirectly through soil acidification (Steubing *et al.* 1989). There may be large variation among trees in responses to air pollutants. This variation can be related to factors such as pollutants' concentration and time of distribution, genetic origin, phenological stage, physiological activity, and nutritional conditions of plants as well as environmental factors (Assadi *et al.* 2011). Leaves are the primary and the most sensitive organ of a tree (Zhou *et al.* 2019). Foliar tolerance to air pollution is usually attributed to the ability of leaves to limit gas exchange ratios (avoidance strategy) (Kolb *et al.* 1997), as well as

to their capacity to activate detoxifying systems (Gerosa *et al.* 2003). In urban environments, trees can absorb and accumulate air pollutants by taking up gases and particles (Woo and Je 2006.). Reducing environmental pollutants by plants is very important and useful. Although the increase in air pollutants may lead to damage to plants, plants resist environmental contamination through various mechanisms (Agbaire and Esiefarienrhe 2009).

The current investigation has been aimed to get more insight into the physiological responses of *U. Minor* to air pollution under different pollutants in Iran.

## Materials and Methods

### Plant materials and pollution data collection

The leaves of *U. minor* Mill. were collected from the Chaloos city in Mazandaran province and three locations in Tehran province including Tajrish, Park Laleh, and Park Shahr. The leaves were randomly collected from the middle part of the crowns of *U. Minor* in each sampling location with five replications (10 leaves from 5 plants in each replication) simultaneously in September 2016. The leaves were transferred to the laboratory at 4 °C. Then the fresh weight was measured. For measurement of the photosynthetic pigments (chlorophyll a, b and carotenoids), anthocyanin, protein content, and peroxidase activity and its isoenzymes, the leaf samples were frozen immediately in liquid N<sub>2</sub> and stored at -80 °C. Also, some leaf samples were air-dried (35 ± 2 °C) for the analysis of dry matter content, total phenols, total nitrogen, and phosphorus content.

The average content of air pollutants such as O<sub>3</sub>, CO, and NO<sub>2</sub> was obtained from the meteorological organization and the air quality control agency of Tehran and Mazandaran provinces that were collected from the nearest reporting stations during five years (2012-2016). Data on climatic conditions including rainfall, temperature, and relative humidity were also obtained from the nearest weather stations during five years (2012-2016).

### Analysis of leaves

#### Chlorophylls and carotenoids content

Chlorophylls and carotenoids were measured according to Lichtenthaler (1987). Fresh leaves were extracted in 80% acetone (1 g 10 ml<sup>-1</sup>). The extract was filtered through Whatman filter paper and centrifuged at 30000 g for 20 min. Then absorbance was measured at 470, 647, and 663 nm for analysis of the chlorophyll a and b and carotenoids, respectively. Pigment concentration was calculated based on mg g<sup>-1</sup> FW.

#### Anthocyanin content

Anthocyanin content was measured by using the method of Masukasu *et al.* (2013). Fresh leaves (0.2 g) were extracted with 3 mL methanol- HCl (V/V HCL 1%). The extract was filtered through Whatman filter paper and centrifuged at 6000 rpm for 20 min at room temperature. The supernatant was kept in a dark place at 4 °C for 24 hours. The absorbance was recorded at 550 nm by a UV-visible spectrophotometer and expressed as μM g<sup>-1</sup> FW.

### **Total phenols content**

Air-dried leaves (0.4 g) were extracted by 10 ml of 70% methanol and sonicated for two hours. Then, the extract was centrifuged at 6000 rpm for 20 min (Thygesen *et al.* 2007). Total phenols content was measured based on a colorimetric oxidant/reduction reaction (Singleton *et al.* 1999). The amount of 125 µl leaf extracts were made up to 500 µl with distilled water, mixed with 2.5 ml 10% Folin for 6 min, and followed by the addition of 2 mL of 7.5% (w/v) sodium carbonate. Then, the absorbance of the leaf extracts was measured at 765 nm after 90 min of incubation at 25 °C in dark conditions. Finally, the total phenols content was determined using a calibration curve of gallic acid and expressed as mg gallic acid equivalents/g dry weight.

### **Leaf nitrogen content**

The nitrogen content of the plants was determined by the method of Kjeldahl (1883). Oven-dried leaves (0.3 g) were digested in sulphuric acid and hydrogen peroxide at 280 °C. Then, sodium hydroxide (2 ml, 10 N), boric acid (15 ml), and Methyl red-bromocresol green indicator were added to the extraction to collect  $\text{NH}_3^+$  by boric acid. In the final step, the amount of ammonia was titrated with sulfuric acid (0.01 N) and continued until the disappearance of green color.

### **Leaf phosphorus content**

Phosphorus content was determined based on vanadate-molybdate reagent (Emami 1996). Oven-dried leaves (1 g) were homogenated with 5 ml of hydrochloric acid (2N) and made up to 50

ml with distilled water. After filtration with Whatman paper, 1 ml of Barton reagent was mixed with 1 ml leaf extract and after 10 min, the intensity of color was measured by spectrophotometer at 470 nm. Finally, the phosphorus content was calculated based on the standard curve of  $\text{KH}_2\text{PO}_4$  on  $\text{mg g}^{-1}$  DW.

### **Protein content**

Frozen leaves were extracted in 0.1 M potassium phosphate buffer with pH 7.0 (1 g fresh weight per 10 ml) at 4 °C and filtered through one layer of Miracloth. This filtered extract was centrifuged at 16,000 g for 25 minutes at 4 °C. The soluble protein content was determined according to Bradford (1976) using bovine serum albumin as the standard solution.

### **Peroxidase activity**

Peroxidase (POD, EC.1.11.1.7) activity was assayed according to Nakano and Asada (1981). The reaction mixture consisted of 200 µl leaf extract, 2.5 ml of potassium phosphate buffer (10 mM; pH 7.0), and 80 µl guaiacol reagent (20 mM). The reaction was started by the addition of 200 µl of  $\text{H}_2\text{O}_2$  (40 mM). Absorbance was recorded at 470 nm after 1 min by a spectrophotometer. The enzyme activity was expressed as  $\text{U mg}^{-1}$  protein.

### **Peroxidase isoenzymes**

Leave extract (40 µl) was mixed with 10 µl of 5X loading buffer containing 250 Mm Tris-HCl, pH 6.8, 10% sodium dodecyl sulfate (SDS), 30% (V/V) glycerol, 0.05% (W/V) bromophenol blue

and loaded to discontinuous 12.5% SDS polyacrylamide gel electrophoresis (SDS-PAGE). Then, the gel was stained for peroxidase activity. To visualize the peroxidase isoenzyme pattern, the gel was incubated in sodium acetate buffer (50 mM; pH 4.5), benzidine hydrochloride (2 mM), and 3 mM H<sub>2</sub>O<sub>2</sub> (Abeles and Biles 1991).

### Statistical analysis

The data were subjected to one-way analysis of variance and then the treatment means were compared by the Tukey's HSD test at  $p \leq 0.01$ . In addition, Pearson correlation coefficients between the variables were calculated. Analyses were carried out using GraphPad Prism (GraphPad Software Inc., USA).

### Results

The five-year weather reports from 2012 to 2016 showed that the average rainfall in Chaloos city was almost 54, 25, and 28 times higher than that in Tajrish, Park Laleh, and Park Shahr in Tehran province, respectively. Likewise, relative humidity in Chaloos city was almost 2 times higher than that in the studied locations in the Tehran province. While the average temperature of different sampling locations did not differ significantly (Table 1).

According to data collected from the air quality control agency, the concentration of O<sub>3</sub>, CO, and NO<sub>2</sub> was lower in the Chaloos location in Mazandaran province than those in Tajrish, Park Laleh, and Park Shahr locations in Tehran province. The concentration of O<sub>3</sub>, CO, and NO<sub>2</sub> in the Park Shahr was almost 2, 2.3, and 5 times

higher than that of the pollutants in Chaloos, respectively (Table 1).

### Contents of dry matter, pigments, elements, and protein

The dry matter content of the leaves of *U. minor* in the sampling location of Park Shahr was approximately 34, 17, and 16 percent higher than that of the leaves of the plants collected from sampling locations Chaloos, Tajrish, and Park Laleh, respectively (Figure 1). In addition, the chlorophyll a and b contents were significantly higher in the sampling locations from Tehran province as compared to the Chaloos location. Both chlorophylls a and b in the leaves of the plants collected from Tehran province were approximately 1.3 times higher than that of the leaves of the plants grown in the Chaloos sampling location. The content of carotenoids and total nitrogen were hardly changed in the leaves of the plants grown in different sampling locations (Figure 1). While the content of phosphorus was dramatically increased with increasing the content of air pollutants. The phosphorus content in the leaves of plants collected from sampling locations of Tajrish, Park Laleh, and Park Shahr was approximately 5 times higher than that of the leaves of the plant collected from Chaloos (Figure 1).

The protein content in the leaves of the plant grown in Park Shahr 4 was almost 3, 1.9, and 1.2 times higher than that of the leaves of the plants grown in sampling locations Chaloos, Tajrish, and Park Laleh, respectively (Figure 1).

Table 1. Longitude, latitude, climate conditions including temperature (°C), rainfall (mm), humidity (%), and the average of 5 years of concentrations of air pollutants such as ozone (ppb), carbon monoxide (ppb), and nitrogen dioxide (ppb) in four sampling locations of Iran.

Plant sampling locations	Chaloos	Tehran-Tajrish	Tehran-Park Laleh	Tehran-Park Shahr
Longitude	36.67067	35.7952	35.7101	35.6830
Latitude	51.24064	51.4323	51.3936	51.4141
Temperature (°C)	18.4 ± 1.6 a	15.6 ± 1.9 a	16.4 ± 1.4 a	17.9 ± 0.7 a
Rainfall (mm)	756 ± 86 a	14 ± 11 b	30 ± 6 b	27 ± 16 b
Humidity (%)	77 ± 8 a	34 ± 9 b	40 ± 5 b	35 ± 7 b
Ozone (ppb)	11±2 b	15±2 ab	16±3 ab	22±3 a
Carbon monoxide (ppb)	1199±172 c	2280±140 b	2060±164 b	2810±203 a
Nitrogen dioxide (ppb)	15±4 c	35±14 b	69±9 a	77±12 a

Means with different letters in each row are significantly different at  $p \leq 0.05$ .

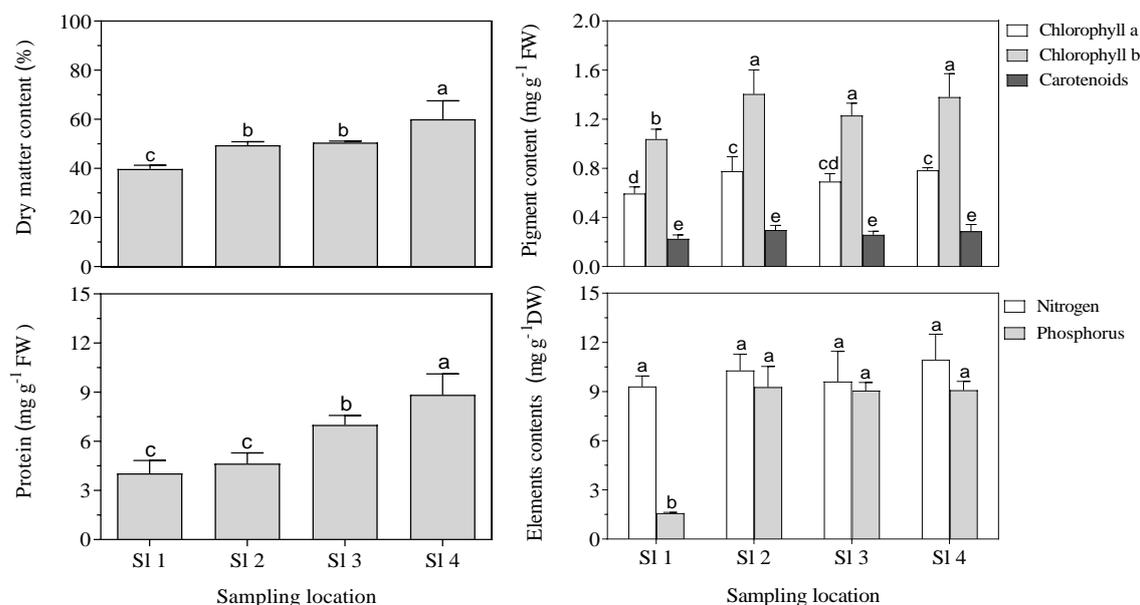


Figure 1. The contents of dry matter, pigments, elements, and protein in the leaves of *U. minor* grown in four different sampling locations; Values with different letters within each character are significantly different at  $p \leq 0.05$ .

### Antioxidative compounds (phenols and anthocyanin)

The content of phenols in the leaves of plants from the sampling locations in Tehran was higher than that of the leaves of plants grown in Chaloos. The content of phenols in the leaves of plants collected from sampling locations Park Shahr and Park Laleh was approximately 10 and 3 percentages higher than that of the leaves of the

plants grown in Chaloos (Figure 2). The anthocyanin content was significantly increased with increasing the ozone content, nitrogen dioxide, and carbon monoxide. The results showed that the anthocyanin content in the leaves of the plants grown in Tajrish, Park Laleh, and Park Shahr was approximately 1.2, 1.4, and 1.5 times higher than that of the leaves of plants from Chaloos (Figure 2).

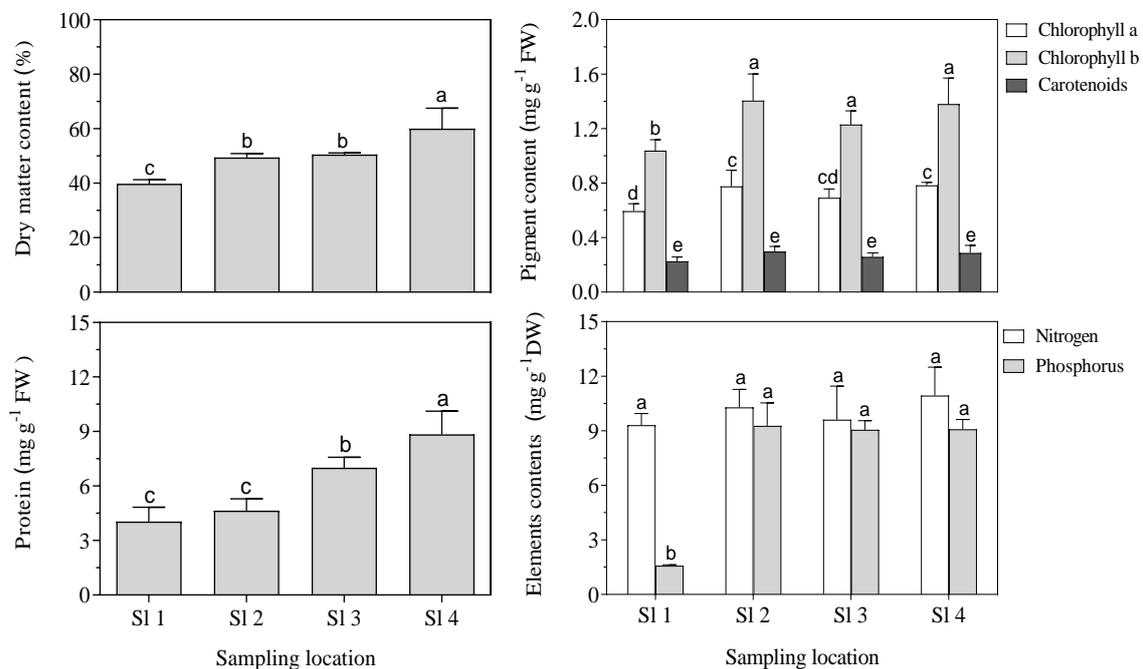


Figure 2. The phenols and anthocyanin content of the leaves of *U. minor* Mill grown in different sampling locations. Values with different letters within each character are significantly different at  $p \leq 0.05$ .

### Peroxidase activity and isoenzymes

The activity of the peroxidase enzyme increased by the increase in the concentration of air pollutants. Peroxidase activity in leaves of the plant collected from Park Shahr, Park Laleh, and Tajrish was almost 16, 16, and 5 percent higher than that of the plants grown in Chaloos (Figure 3 A).

The peroxidase isoenzyme pattern showed two bands (b and d) in the plants from Chaloos, three bands (b, c, and d) in the plants collected from Tajrish and Park Laleh, and four bands (a, b, c, and d) in plants from sampling location of Park Shahr. In addition,

the intensity of all bands was increased with increasing the air pollutants in the sampling locations (Figure 3 B).

### Correlation among variables

The results showed that CO and O<sub>3</sub> were directly correlated with dry matter content and pigments content while NO<sub>2</sub> did not show any correlation with the pigment content. However, all air pollutants (CO, O<sub>3</sub>, and NO<sub>2</sub>) were positively correlated with the dry matter content, protein, anthocyanin, and phosphorous (Table 2).

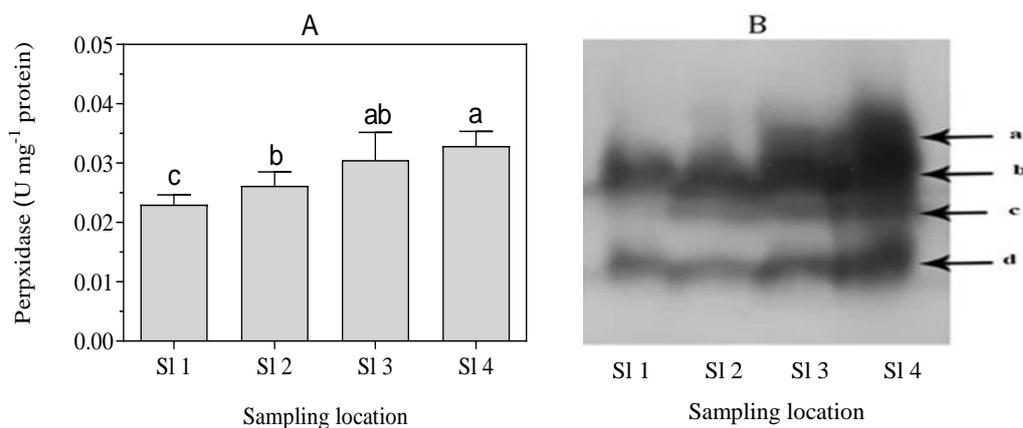


Figure 3. Peroxidase activity (A) and peroxidase isoenzyme pattern (B) of the leaves of *U. minor* Mill grown in four different sampling locations.

Table 2. Pearson correlation coefficients of atmospheric pollutants with physiological and biochemical characters of the *Ulmus minor* leaves.

Variables	Dry matter	Chl a	Chl b	Carotenoids	Protein	Anthocyanin	Phenol	Peroxidase	N	P
Carbon monoxide	.969**	.680**	.696**	.601**	.744**	.873**	-.545*	.167	.398	.945**
Ozone	.974**	.572**	.586**	.493*	.863**	.789**	-.574**	.239	.377	.869**
Nitrogen dioxide	.897**	.400	.425	.337	.906**	.597**	-.832**	.334	-.013	.836**

\*, \*\*Significant at 0.05 and .01 probability levels, respectively; Chl: Chlorophyll

## Discussion

The current study showed that CO and O<sub>3</sub> were strongly and positively correlated with the pigments content and dry matter content (Table 2). The increase in the content of both chlorophylls a and b in the leaves of *U. minor* in different sampling locations in Tehran can be considered as one of the important factors in preserving photosynthetic capacity under the air pollution stress (Jiang and Huang 2001). It has been shown that the thickness of the leaves is usually increased in the contaminated areas (Gratani *et al.* 2000) and the thick leaves have higher photosynthetic activity (Niinemets 1999), which in turn can increase the amount of photosynthetic

pigmentation such as chlorophyll a and b. However, it seems there is a limitation in the biosynthesis of both pigments when the leaves of *U. minor* are exposed to O<sub>3</sub>, CO, and NO<sub>2</sub> at concentrations higher than 15.8, 2060, and 35 ppb, respectively. Likewise, an increase in the leaf dry matter content as a growth factor is the result of the increase in the content of photosynthetic pigments (Figure 1) and consequently an increase in the photosynthetic product under air contamination (Joshi and Swami 2009). However, carotenoids as an auxiliary pigment and antioxidant which protect the photosynthetic pigments from intense and destructive light (Murchie and Niyogi 2011), was remained unaffected in

both provinces with high (Tehran) and low (Mazandaran) air pollutants (Figure 1).

Interestingly, an increase in the biosynthesis of photosynthetic pigments in the leaves was accompanied by the accumulation of the phosphorus element in the leaves (Figure 1) which can be justified by the role of this element in photosynthesis and regulating the Calvin cycle (Fredeen *et al.* 1990). The higher accumulation of phosphorus in plants in the areas with higher air pollution may lead to the stimulation of more protein synthesis under stress to increase plant resistance due to the importance of phosphorus as an essential element in the process of protein biosynthesis (Malhotra *et al.* 2018). Phosphorus also has a role in the cell structure and catalytic function of enzymes involved in metabolism (Akhtar *et al.* 2009). An increase in the protein content of the leaves of *U. minor* collected from different sampling locations in Tehran under the stress of all studied air pollutants ( $O_3$ , CO, and  $NO_2$ ) (Figure 1), may indicate the role of protein in regulating osmotic pressure, the involvement of the protein in the structure of oxidative enzymes or as a source of nitrogen that is renewable (Timperio *et al.* 2008).

The anthocyanins and phenols (Figure 2) and the activity of the peroxidase enzyme and the number and intensity of its isoenzymes (Figure 3) increased in the leaves of *U. minor* collected from the locations in Tehran with high air pollutants. However, only anthocyanins showed a significant positive correlation with air pollutants ( $O_3$ , CO, and

$NO_2$ ) especially with CO (Table 2). It seems high concentrations of air pollutions lead to excessive accumulations of reactive oxygen species (ROS) which can subsequently attack bio-macromolecules including proteins, DNA, and lipids (Juan *et al.* 2021). Plants remove the free radicals by enzymatic and non-enzymatic antioxidant systems which are regulated by a ROS-mediated signaling pathway (Oskuei *et al.* 2013).

In the current study, induction of new isoenzymes and increase in the intensity of isoenzyme bands in conditions of high pollutants, confirm the increase in plant resistance through the defensive role of the peroxidase enzyme (Figure 3). Peroxidase is an important catalyst that plays a major role in most metabolic reactions (Iranmanesh *et al.* 2009).

The continuous increase in the human population, industrialization, and pollutant emissions by traffic has increased the concentration of particulate and gaseous pollutants like  $O_3$ , CO, and  $NO_2$  in developed cities including Tehran as compared to Chaloos city in Mazandaran province with relatively low pollution (Table 1). In the current study a positive correlation of CO with anthocyanin and phenol has been observed (Table 2).

Studies have shown that high concentrations of CO cause the rapid generation of hydrogen peroxide and singlet oxygen and thus activate the response pathways of the ascorbate peroxidase cycle as

well as an increase in the content of phenolic compounds and the activity of antioxidant enzymes like catalase, peroxidase, and superoxide dismutase (Muneer *et al.* 2014). In addition, CO at high concentration in the polluted area has an inhibitory effect on cytochrome c oxidase in the electron transport chain in the mitochondria and the effect on the detoxification enzyme P-450 in the monooxygenase system (Zuckerbraun *et al.* 2007; Muneer *et al.* 2014).

The current study showed that the exposure of the leave to O<sub>3</sub> led to an increase in the content of phenolic compounds probably due to an increase in the activity of the phenylalanine ammonia-lyase and chalcone synthetase (Francini *et al.* 2008). The O<sub>3</sub> directly reacts with the plasmalemma through ozonolysis or through converting it into ROS which reacts with the susceptible amino acids in membrane proteins or apoplastic enzymes, plasmalemma, and also several organic metabolites in the cell wall (Flowers *et al.* 2007). Therefore, an increase in the antioxidant content in the polluted areas with a high content of O<sub>3</sub> would be expected to remove the ROS.

An increase in the content of antioxidants in the areas with higher NO<sub>2</sub> concentrations (Figure 2) can be due to their capacity to scavenge free radicals in plants grown in contaminated areas. The high concentrations of NO<sub>2</sub> can also result in cell acidification (Schmutz *et al.* 1995) and excessive accumulation of nitrite (Okano and Totsuka 1986), which subsequently leads to the

generation of ROS (Sheng and Zhu 2019).

Climate factors such as rainfall and humidity were related to pigments, dry matter content, and anthocyanin contents (Table 1). An increase in the content of pigment following the reduction of the water access to the plants in different sampling locations in Tehran province may be due to an increase in chloroplast functionality or adaptation to the osmotic stress (Locy *et al.* 1996).

### Conclusion

In general, the most physiological defensive reactions of leaves were associated with CO and O<sub>3</sub> but not NO<sub>2</sub>. In addition, *U. minor* can be considered as a tree resistant to air pollution due to an increase in the content of antioxidant compounds such as phenols and anthocyanin, increase in activity of peroxidase, and intensity of its isoenzyme bands and increase in the content of protein and pigments. Therefore, we can recommend the planting of *U. minor* as a biological filter in industrial areas like Tehran city to remove significant amounts of dust from urban atmospheres and improve air quality.

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### Conflict of Interest

The authors declare that they have no conflict of interest with any organization concerning the subject of the manuscript.

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## پاسخ‌های فیزیولوژیکی نارون به ازن، مونوکسید کربن و دی‌اکسید نیتروژن در مناطق با سطح آلاینده‌های اتمسفری مختلف در ایران

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### چکیده

مطالعه حاضر با هدف بررسی پاسخ‌های فیزیولوژیکی برگ‌های درخت نارون (*Ulmus minor* Mill.) به آلاینده‌های هوا مانند ازن، مونوکسید کربن و دی‌اکسید نیتروژن به منظور بررسی توانایی مقاومت این گونه در برابر آلاینده‌های هوا انجام شد. برگ‌های نارون از یک مکان در استان مازندران (با آلودگی هوای کمتر) و سه مکان در استان تهران (با آلودگی هوای بالاتر) جمع‌آوری گردید. برگ‌ها به طور تصادفی از قسمت میانی تاج‌ها در هر مکان نمونه برداری با پنج تکرار جمع‌آوری شد و رشد و صفات فیزیولوژیکی اندازه‌گیری گردید. فاکتورهای آب و هوایی مکان‌های نمونه برداری نیز مورد ارزیابی قرار گرفت. نتایج نشان داد که محتوای ماده خشک، پروتئین، کلروفیل a، آنتوسیانین و فعالیت آنزیم پراکسیداز در برگ‌های گیاهان رشد یافته در منطقه ۴ با مقدار بالاتر آلاینده‌های هوا به ترتیب، تقریباً ۳۵، ۵۰، ۳۰، ۵۰ و ۴۰ درصد بیشتر از مقدار آن‌ها نسبت به برگ‌های گیاهان جمع‌آوری شده در منطقه ۱ با میزان کمتر آلودگی هوا بود. تعداد و شدت باندهای ایزوآنزیم پراکسیداز در برگ گیاهان جمع‌آوری شده از منطقه نمونه برداری در استان تهران به طور قابل توجهی بیشتر بود. با این حال، نقش برجسته بارندگی در پاسخ‌های فیزیولوژیکی نارون را نمی‌توان نادیده گرفت. به نظر می‌رسد که نارون درختی مقاوم در برابر آلاینده‌های هوا می‌باشد و ممکن است به عنوان یک فیلتر بیولوژیکی در حذف آلاینده‌های گازی در مناطقی مانند شهر تهران در نظر گرفته شود تا کیفیت هوا بهبود یابد.

واژه‌های کلیدی: آلودگی هوا؛ آنتی‌اکسیدانت‌ها؛ عوامل آب و هوایی؛ مقاومت؛ نارون